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NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

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in its capacity as elected Office

Date of mailing (day/month/year) 03 February 1999 (03.02.99)	
International application No. PCT/GB98/01882	**Applicant's or agent's file reference** JDH/GCN/2171PC
International filing date (day/month/year) 26 June 1998 (26.06.98)	**Priority date** (day/month/year) 26 June 1997 (26.06.97)
Applicant THOMSON, Brian, Mark et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

21 December 1998 (21.12.98)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

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(71) Applicant *(for all designated States except US)*: SMITH & NEPHEW PLC [GB/GB]; 2 Temple Place, Victoria Embakment, London WC2R 3BP (GB).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: THOMSON, Brian, Mark [GB/GB]; 33 Burnby Lane, Pocklington, York YO42 2QE (GB). ALI, Saad, Abdul, Majeed [GB/GB]; 71 Yarburgh Way, York YO10 5HQ (GB). MEDCALF, Nicholas [GB/GB]; 12 Clayfield Close, Pocklington, York YO42 2PU (GB). MALTMAN, John [GB/GB]; 12 Lundy Close, Waterside Park, Clifton, York YO30 5GQ (GB). WINTER, Sharon, Dawn [GB/GB]; 12 Lundy Close, Waterside Park, Clifton, York YO30 5GQ (GB).

(74) Agent: SMITH & NEPHEW GROUP RESEARCH CENTRE; Group Patents & Trade Marks Dept., York Science Park, Heslington, York YO10 5DF (GB).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

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Without international search report and to be republished upon receipt of that report.

(54) Title: CELL CULTURE PRODUCTS

(57) Abstract

A wound dressing which comprises a carrier layer having a non–adherent to cell layer on a wound facing surface thereof. The non–adherent layer has bonded thereto a biodegradable cell anchoring layer which anchors mammalian cells. In use, the degradable layer breaks down releasing the cells into the wound site which are discouraged from reattaching to the dressing by the non–adherent layer. Thus the dressing can switch from a cell binding state to a state in which the binding of cells is discouraged. Systems, methods of treatment and methods of manufacturing the dressing are also disclosed.

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PATENT COOPERATION TREATY

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INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
JDH/GCN/2171PC		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/GB 98/ 01882	26/06/1998	26/06/1997
Applicant		
SMITH & NEPHEW PLC et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. [ ] **Certain claims were found unsearchable** (see Box I).

2. [ ] **Unity of invention is lacking** (see Box II).

3. [ ] The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing
   - [ ] filed with the international application.
   - [ ] furnished by the applicant separately from the international application,
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   - [ ] Transcribed by this Authority

4. With regard to the **title,**
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   - [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**
   - [X] the text is approved as submitted by the applicant
   - [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this International Search Report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:

   Figure No. _____
   - [ ] as suggested by the applicant.
   - [ ] because the applicant failed to suggest a figure.
   - [ ] because this figure better characterizes the invention.

   [X] None of the figures.

INTERN NAL SEARCH REPORT

Inter al Application No
PCT/GB 98/01882

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61L15/40 A61L15/22 A61L27/00 C12N5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 06837 A (INTEGRA LIFESCIENCES CORP) 27 February 1997	1,2
Y	see page 1, line 12 - line 24	3,17-19
A	see page 2, line 9 - line 20	4-9, 12-14
	see page 8, line 7 - line 17	
	see page 12, line 29 - page 13, line 17	
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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Date of the actual completion of the international search	Date of mailing of the international search report
11 January 1999	19/01/1999

Name and mailing address of the ISA	Authorized officer
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Form PCT/ISA/210 (second sheet) (July 1992)

Inter al Application No
PCT/GB 98/01882

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 97 06835 A (SMITH & NEPHEW ;RICHARDSON MARK CHRISTOPHER (GB); BLOTT PATRICK LE) 27 February 1997 cited in the application	3,17-19
A	see page 3, paragraph 2 see page 5, line 3 - line 9 see page 7, paragraph 3 see page 11, paragraph 1 see page 12, paragraph 1 see page 17; claims 1,5,9,16-19,22	1,2,5, 12-15
X	US 4 060 081 A (YANNAS IOANNIS V ET AL) 29 November 1977	1,2
A	see column 6, line 10 - column 7, line 44 see column 13, line 47 - column 14, line 4	3-7, 12-16
A	US 5 410 016 A (HUBBELL JEFFREY A ET AL) 25 April 1995 see column 4, line 29 - line 39 see abstract; claims 1,19	1-8,10, 11

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 98/01882

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(71) Applicant: INTEGRA LIFESCIENCES CORPORATION [US/US]; 105 Morgan Lane, P.O. Box 688, Plainsboro, NJ 08536 (US).

(72) Inventor: CAHN, Frederick; Apartment 10, 113 Cascade Court, Princeton, NJ 08540 (US).

(74) Agents: CARROLL, Alice, O. et al.; Hamilton, Brook, Smith & Reynolds, Two Militia Drive, Lexington, MA 02173 (US).

(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

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(54) Title: PERFORATED ARTIFICIAL SKIN GRAFTS

(57) Abstract

Disclosed is a perforated multilayer membrane useful as artificial skin. The multilayer membrane comprises a porous biodegradable polymeric membrane having a moisture control layer disposed thereon. The moisture control layer is perforated such that the multilayer membrane is permeable to fluid in the presence of hydrostatic pressure from exudate in the wound while being substantially impermeable to fluid and water vapor in the wound in the absence of hydrostatic pressure from exudate in the wound. Also disclosed is a method of covering a burn or wound with the perforated multilayer membrane.

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PERFORATED ARTIFICIAL SKIN GRAFTS

Background

Each year there are approximately two million patients with burns requiring medical attention in the United States. Of these injuries, there are roughly 130,000 hospital admissions, of which about 20,000 are considered life-threatening. Successful treatment requires rapid covering of the burn wound. The wound cover of choice is conventional autograft; however, burn wound management is frequently hampered by the lack of availability of a suitable quantity of donor skin from the patient.

Recent advancements in burn treatment have made use of artificial skin. One of the more successful is a bilayer membrane (Yannas *et al.*, U.S. Patent 4,060,081). The bilayer membrane comprises a first layer formed from a crosslinked collagen-glycosaminoglycan composite and a moisture transmission control layer formed from a nontoxic material. The moisture transmission control layer provides the multilayer membrane with a controlled moisture flux. The multilayer membrane not only provides immediate wound closure, but also builds neodermis, thus permitting the satisfactory use of a thin epidermal autograft (or cultured epidermal cells) rather than a thick conventional autograft. It also results in less hypertrophic scar formation, thereby yielding cosmetic outcomes comparable to or better than conventional autograft techniques.

The use of bilayer membranes and other temporary wound coverings is compromised by the high rate of infections associated with their use. The control of infection in burn wounds covered with the bilayer membrane or other temporary coverings would significantly advance the ability to successfully treat patients with severe and extensive burns.

Summary of the Invention

The present invention is based on the unexpected discovery that perforations (also referred to herein as "meshings") in multilayer membranes, used as synthetic skin to repair burn wounds, can significantly reduce the incidence of infection at the wound site and also increase the extent at which the graft will adhere to or "take" to the wound.

One embodiment of the present invention is a multilayer membrane useful as artificial skin. The multilayer membrane comprises a porous biodegradable polymeric membrane having a moisture control layer disposed thereon. The porous biodegradable polymeric membrane typically has (1) controllable biodegradability in the presence of body enzymes; (2) has controllable solubility in the presence of bodily fluids; (3) is substantially nonimmunogenic upon grafting or implantation; (4) provokes no substantial foreign body response upon grafting or implantation; and (5) promotes the adherence and proliferation of cells, such as fibroblasts and endothelial cells. The moisture control layer is perforated such that the multilayer membrane is permeable to fluid in the presence of hydrostatic pressure from exudate in the wound while being substantially impermeable to fluid and water vapor in the wound in the absence of hydrostatic pressure from exudate in the wound.

Another embodiment of the present invention is a method of covering a full thickness or partial thickness burn or other wound site on a human or animal. The method comprises applying the multilayer membrane described above to the burn or wound site.

The perforations in the multilayer membrane allow pus and exudate to drain from the wound site while still providing the moisture transmission control layer with sufficient moisture impermeability to prevent significant moisture loss from the wound. Use of perforated multilayer

membranes to treat burn wounds leads to significantly lower incidence of infection compared with unperforated membranes. When infections occur in wounds covered by a perforated multilayer membrane, they are generally of reduced severity. Perforated multilayer membranes also "take", i.e. adhere to and become permanently fixed to the wound bed, more completely than multilayer membranes which lack the perforations.

Brief Description of the Drawings

Figure 1 illustrates a non-expandable multilayer membrane with a multiplicity of aligned, non-overlapping slit perforations.

Figure 2 illustrates an expandable multilayer membrane with a multiplicity of staggered, overlapping slit perforations.

Figure 3 illustrates a multilayer membrane with a multiplicity of cross-slitted perforations arranged in a rectangular pattern.

Figure 4 illustrates a multilayer membrane with a multiplicity of hole perforations arranged in a trigonal pattern.

Figure 5 illustrates schematically a multilayer membrane as described herein, wherein the perforations are not shown.

Detailed Description of the Invention

Moisture transmission control layers, such as those described in U.S. Patent No. 4,060,081, have been used in artificial skin to control the rate of body moisture loss and heat loss from the damaged skin area. Although this layer is important in homeostasis and protecting the wound area from mechanical abrasion, it can also trap exudate from the wound. Infection or exudate can decrease the ability of an artificial skin graft to "take" to the wound

site, i.e. to adhere to the wound. Infections develop and spread because wound exudate and pus in infected areas is trapped under the moisture transmission control layer and thus spread laterally. The present invention provides a means for significantly reducing and/or eliminating this type of infection. It has been discovered that if the multilayer membrane is perforated, exudate and pus can drain away from the wound site and relieve hydrostatic pressure. Typically, the exudate is absorbed by absorbent dressings which are used to cover the artificial skin.

The perforations are constructed in a manner to substantially prevent the passage of fluids and water vapor in the absence of hydrostatic pressure. The perforations in the multilayer membrane have a size and shape and are arranged in a pattern such that the membrane is permeable to fluid in the presence of hydrostatic pressure from exudate in the wound. The size, shape and pattern of the perforations are chosen so that the moisture loss from a wound to which the multilayer membrane has been applied is maintained below about 2.2 $mg/cm^2/hour$, and preferably between about 0.1 $mg/cm^2/hour$ and about 1.0 $mg/cm^2/hour$.

The perforations are preferably spaced as close together as possible to minimize the lateral path for exudate to spread within the wound site. The total open area of the perforations is preferably small to minimize moisture loss in the absence of hydrostatic pressure, and also to maximize the resistance to fluid flow at low hydrostatic pressures. The perforations penetrate completely through the moisture transmission control layer. Optionally, the perforations penetrate either partially or completely through the porous biodegradable polymeric membrane layer. However, it is preferred that only the moisture transmission control layer be completely penetrated by the perfora-

tions. Perforations are also referred to herein as "meshings".

In a preferred embodiment, the perforations comprise a multiplicity or plurality of slits. The slits can be arranged in a wide variety of patterns. It is preferred that the slit pattern results in a non-expandable multilayer membrane. Alternatively, the slit pattern can result in a multilayer membrane which expands upon the application of a lateral force to the multilayer membrane. An expandable multilayer membrane can be stretched along at least one lateral axis. A "lateral axis" is an axis or line which traverses the entire cross section of a multilayer membrane. When an expandable membrane is pulled in opposite directions at each end of the lateral axis, the membrane is stretched, thereby resulting in an increased surface area. An increase in surface area has the undesired effect of pulling the slits open to form permanent openings which will expose the underlying wound to increased moisture loss and external pathogens. Thus, it is essential to maintain the integrity of the membrane to optimize the proper function of the moisture control barrier. Consequently, an expandable membrane is applied to a wound and maintained under conditions which prevent expansion or increases in the surface area of the membrane.

A membrane which does not stretch or increase its surface area when pulled in opposite directions along a lateral axis is said to resist expansion along that lateral axis. A non-expandable multilayer membrane resists expansion along any lateral axis.

A multilayer membrane having a multiplicity of slits resists expansion along a lateral axis when the lateral axis is not intersected by any slit. As a consequence, this type of multilayer membrane has a continuous band of membrane along the lateral axis that is free of slits. A non-expandable multilayer membrane has at least two perpen-

dicular continuous bands of membrane in which the slits do not intersect. As a result, the membrane can be pulled in opposite directions along any lateral axis without stretching or increasing the surface area of the multilayer membrane.

One example of a multilayer membrane which resists expansion is shown in Figure 1. The slits are arranged in non-overlapping parallel rows with the slits in each row being parallel to one another. A continuous band of membrane in which the slits do not intersect runs the length of the membrane. Preferably, the slits are of equal length, are aligned with the slits in the same row, and are aligned with a slit in the adjacent row. Dimensions a, b and c in Figure 1 typically range from about 0.5 mm to about 5.0 mm in length. In one example, a is 1.7 mm, b is 1.1 mm and c is 2.7 mm.

A multilayer membrane is expandable along a first lateral axis when at least one slit intersects each lateral axis parallel to the first lateral axis. In a preferred example of an expandable multilayer membrane, the slits are arranged in non-overlapping parallel rows with the slits in each row being parallel to one another. The slits are of equal length and staggered with respect to the other slits in the row such that there is no continuous band of membrane between the rows which runs the length of the multilayer membrane sample. As a result, the membrane will expand when opposing forces are applied at opposite ends of a lateral axis running perpendicular to the slits. This type of expandable membrane is shown in Figure 2. The degree of expansion is controlled by the length of the slits and the number of overlapping slits. Expandable membranes can be stretched, for example, to about 1.5, 2.0, 2.5 and 3.0 times their normal surface area.

In another embodiment the perforations comprise a plurality of cross-slits. Cross-slits can open like a

valve, thereby maximizing fluid flow away from the wound site. As described above, the cross-slits can be arranged so that the membrane is expandable or is non-expandable. The cross-slits can be arranged into regular or irregular patterns, however regular patterns in which the slits are evenly spaced are preferred. Suitable patterns include polygonal patterns such trigonal, rectangular (see Figure 3) and hexagonal patterns.

In another embodiment the perforations comprise a plurality of holes. The holes are of sufficient size so that they do not clog, but are not so large that excessive amounts of fluid or water vapor escape from the wound. Suitable diameters of the holes are from about 100 microns to about 2 millimeters. As described for the cross-slits, holes can be arranged in irregular or regular patterns, however, regular patterns in which the holes are evenly spaced are preferred (see Figure 4; trigonal pattern shown).

The perforated multilayer membranes and synthetic skins of the present invention are prepared by meshing conventional multilayer membranes and artificial skins with meshing machines such as a Collins ampligraft or a Brennen mesher or a similar device used to prepare meshed autograft. A 1:1 meshing ratio is preferred. The same operational technique to prepare meshed autograft may be used. Any other mechanical means for spacing the perforations in the patterns described herein can be used.

Multilayer membranes and synthetic skins are well known in the art and are disclosed in Yannas *et al.*, U.S. Patent No. 4,060,081, Yannas and Kirk, U.S. Patent No. 4,448,718 and Yannas *et al.*, U.S. Patent No. 4,947,840, the teachings of which are hereby incorporated into this application in their entirety. These and other multilayer membranes and synthetic skins suitable for perforation and use in the present invention are described below. It is to

be understood that there are many modifications which the skilled artisan could make to synthetic skin and multilayered membranes without affecting its suitability for use in the present invention. Many of these modifications are presently known in the art while others may be developed in the future. Such modifications are encompassed within the scope of the present invention. The multilayer membranes described herein have at least two layers of different materials. As illustrated in Figure 5, there is a first layer **10**. Since the first layer **10** comes into direct contact with the subcutaneous tissue or wound bed, there are three essential characteristics required of this layer. These are: insolubility in body fluids; ability to promote the adherence and proliferation of cells, such as fibroblasts and endothelial cells; a controlled rate of biodegradation such that the material provides a scaffold suitable for wound repair; and nonimmunogenicity. These multilayer membranes also include at least one additional layer, which has the primary function of controlling the moisture flux for the overall membrane. Thus, moisture transmission control layer **12** is illustrated in Figure 5 as being directly bonded to the first layer. It should be understood, however, that additional layers can be added on top of layer **12** or between first layer **10** and moisture control layer **12** as long as such additional layers do not interfere with the essential functions of layers **10** and **12**.

The first layer is preferably a porous biodegradable polymeric membrane layer comprising a composite formed from collagen molecules that are crosslinked and covalently bonded with glycosaminoglycan (GAG). Examples of specific glycosaminoglycans include but are not limited to chondroitin 6-sulfate, chondroitin 4-sulfate, heparan, heparan sulfate, keratan sulfate and dermatan sulfate. Also, other anionic polymers such as chitin and chitosan are suitable.

In a preferred embodiment of this invention, the average pore size of the biodegradable first layer is within the range of about 9 μm to about 630 μm, preferably about 20 μm to 200 μm. The average pore size can be calculated by stereology from scanning electron micrograph of the surface or cross section as described by Dagalakis *et al. J. of Biomedical Materials Research 14*:511 (1980). Materials which do not come within these parameters do not delay or arrest skin wound contraction and thus tend to induce synthesis of undesirable scar tissue, while those materials having pore sizes within the desired upper and lower limits have been found to effectively delay or arrest skin wound contraction and induce synthesis of new functional tissue. Another determining factor in the effectiveness of multilayer membranes is the pore volume fraction of the first layer. This value is defined as the percentage of the total volume of the material which is occupied by pore space. A more detailed definition is given in Fischmeister, H.F. Proceedings Int. Symp. RILEM/IUPAC, Prague, September 18-21, 1973, Final Report Part II, p. C-439, the entire teachings of which are incorporated herein by reference. A high pore volume fraction in the first layer has been found to be clinically desirable, with pore volume fractions above about 80% being preferred.

The degree of crosslink density is an important parameter of this invention since it is a direct, controlling factor in the biodegradation rate of the material. Generally, the greater the crosslink density, the lower the degradation rate, and vice versa. By controlling the degree of crosslinking, composites can be produced which exhibit a degradation rate within a range determined to be clinically desirable. The maximum degradation rate has been determined to be about 140 enzyme units (e.u.), and is preferably below about 120 e.u. The crosslinked composites should have an average molecular weight between crosslinks,

(M_c), of between about 800 and about 60,000 daltons. Composites with an M_c of between 10,000 and about 40,000 tend to have the best balance between physical and therapeutic properties and are this preferred.

A preferred method for covalently crosslinking the collagen-GAG composites is known as aldehyde crosslinking. In this process, the materials are contacted with aqueous solutions of aldehyde, which serve to crosslink the materials. Suitable materials include formaldehyde, glutaraldehyde and glyoxal. Glutaraldehyde is preferred because it yields the desired level of crosslink density more rapidly than other aldehydes and is also capable of increasing the crosslink density to a relatively high level. Composites suitable for use in the present invention can be made by forming an uncrosslinked material comprising a reaction product of collagen and a glycosaminoglycan and contacting the reaction product with an aqueous glutaraldehyde solution for a period in excess of one hour. The resulting crosslinked collagen-glycosaminoglycan composite has a rate of biodegradation which is low enough to enable the composite to be a suitable scaffold for wound repair. The maximum degradation rate has been determined to be about 140 enzyme units (e.u.), measured as described in Yannas *et al.*, U.S. Patent No. 4,947,840. Preferably, the biodegradation rate is below about 120 e.u.

It is preferred that the collagen quaternary structure after glutaraldehyde cross-linking be unbanded. Unbanded structures are characterized by the absence of periodic banding at 640Å, characteristic of native collagen, when viewed by transmission electron microscopy (Sylvester *et al.*, *Thrombosis Research* 55:135 (1989)). Unbanded structures can be obtained by crosslinking at pHs below about 4.25, preferably at about 3.0.

Covalent crosslinking can be achieved by other specific techniques including radiation and dehydrothermal methods. An example of a suitable crosslinking technique is to treat collagen with 0.25% aqueous glutaraldehyde solution in 0.05 M acetic acid for twenty four hours at 20-25°C. These techniques are discussed in greater detail in Yannas *et al.*, U.S. Patent No. 4,060,081, Yannas and Kirk, U.S. Patent No. 4,448,718 and Yannas *et al.*, U.S. Patent No. 4,947,840, the entire teachings of which have been incorporated herein by reference. Other suitable chemical crosslinking techniques include carbodiimide coupling, azide coupling and diisocyanate crosslinking.

Particularly preferred first layer materials are crosslinked collagen-glycosaminoglycan composites containing between about 6% and about 15% of a sulfate-containing mucopolysaccharide and crosslinked to an M_c value of between about 5,000 and about 10,000. Chondroitin 6-sulfate forms especially outstanding composites.

A moisture transmission control layer is formed from a material which provides the moisture flux per unit area described above. These values are obtained by an appropriate combination of thickness, water transmission properties and the size, shape and pattern of the perforations. It is been found that a bilayer membrane produced as described in Example 1 and perforated through both the silicone and collagen GAG layer with a Brennen 1:1 mesher to give the slit pattern of Figure 1 showed no increase in vapor permeability (n=4) (0.65 ± 0.04 mg/hr/cm^2) compared with unperforated material (0.64 ± 0.02 mg/hr/cm^2). A bilayer membrane with perforations that cut the silicone layer, but only partially penetrated collagen-GAG, prepared with a modified Brennen mesher (1.236 inch diameter roller), operated with the silicone side of the membrane towards the

cutting blade, showed essentially the same vapor permeability (0.64 mg/hr/cm^2).

The other essential property of this layer is that it be nontoxic. The material should contain no toxic substances capable of diffusing out into tissues contacting a multilayer membrane graft or capable of being extracted therefrom. Also, the material should be capable of resisting enzymatic degradation or other degradation resulting from contact with other layers of the membrane or with tissue which degradation might lead to the production of substances that are toxic to neighboring tissue.

As is the case with the first layer, there are several other desirable properties for the layer which primarily controls the moisture flux. Thus, it is desirable that the moisture-control layer adhere to the wet surface of the first layer with a bond shear strength of at least about 10 psi, and preferably about 100 psi. It also is desirable that it have mechanical properties of: Young's modulus in the range of from about 100 to 1,000 psi; ultimate tensile strength of from about 100 to about 1,000 psi; and elongation at break of from about 20 to about 100%

Additionally, it is advantageous if the moisture control layer is capable of being sterilized, i.e., of being subjected to physical or chemical treatment that kills bacteria and bacterial spores on its surface. Suitable sterilization techniques include dry heat, exposure to ethylene oxide, irradiation, immersion in glutaraldehyde solution, etc.

Synthetic polymeric materials which can be used in the moisture control layer include: silicone polymers, such as Silastic Medical Adhesive (Dow Corning), a mixture of an hydroxyl terminated silicone polymer and methyl triethoxy silane which moisture-cures into a flexible, tough layer that adheres very well to first layer materials such as

crosslinked collagen-glycosaminoglycan composites; polyacrylate or polymethacrylate esters or their copolymers such as an acrylic rubber latex formed from an ethyl acrylate-acrylic acid copolymer which forms a flexible film on top of first layer materials and which contains carboxylic acid groups capable of reacting with hydroxyl groups present in crosslinked collagen-glycosaminoglycan materials to form strong bonds; polyurethanes such as a reaction product of excess toluene diisocyante with a mixture of diols and triols to give a reactive, moisture-curing prepolymer capable of forming an elastomeric layer on crosslinked collagen-glycosaminoglycan composites and having chemical groups which react with amino groups or hydroxyl groups in such composites. Those skilled in the art will recognize or be able to ascertain, using no more than routine experimentation, other materials which are suitable for the moisture control layers.

Silicone polymer is preferred as the moisture control layer. It is available as a non-toxic product in a carefully controlled medical grade. Its flow properties are of thixotropic nature, permitting uniform application by knife blade onto the surface of collagen-glycosaminoglycan composite layer with controlled penetration into the latter. Curing can be done at 100% relative humidity, thereby avoiding dehydration of the lower layer, consisting of the collagen-glycosaminoglycan composite, and preventing deformation of the multilayered structure. Silastic Medical Grade silicone typically has 180° peel strength, between 6 and 16 g/cm.

Multilayer systems can also be made by using a moisture-curing silicone elastomer as the agent bonding the collagen-glycosaminoglycan layer to another material. By applying a thin film (1-2 thousandths of an inch) over a film prepared from synthetic polymers such as the segmented polyurethanes, hydroxyethyl methacrylate and other "hydro-

gels", polyethylene terephthalate and polytetrafluoroethylene or from modified natural polymers such as cellulose acetate or from natural polymers such as elastin (the fibrous, insoluble, noncollagenous protein found in connective tissue such as the thoracic aorta and ligamentum muchae), a multicomponent composite can be obtained by curing at room temperature at 100% relative humidity for 16-24 hours.

If mechanical reinforcement is desired, a layer of gauze or other fabric or mesh could be usefully employed. Cotton or other textile mesh can be incorporated as a reinforcing mechanism by placing the textile material over the collagen-glycosaminoglycan composite and applying the Silastic silicone over the mesh onto the collagen-glycosaminoglycan surface by knife coating. Curing at room temperature and 100% relative humidity overnight (16-24 hours) can result in a reinforced composite which is somewhat stiffer than one without the mesh but with substantially improved tensile strength.

The optimum thickness of a synthetic skin is related to the following parameters: (1) thickness of the skin to be replaced; (2) nature of wound and dimensions; (3) thickness of top layer required to control moisture flux; and, (4) relation of suturability and drapability to thickness.

The lowest attainable limit of thickness for the collagen-glycosaminoglycan layer is dependent upon the particle size of the collagen-glycosaminoglycan composites and is typically in the range of 1.5-2.0 mils. The upper limit of thickness depends only upon the application contemplated and in practice are available up to indefinitely high levels depending upon the quantity of dispersion filtered through a given area of filter. Thickness as high as 100-200 mils are readily prepared by the process described in U.S. Patent Nos. 4,060,081 and 4,947,840, al-

though for application as a skin substitute, the preferred range is 25-100 mils.

On the other hand, the thickness of the top layer would be dictated by the desired moisture flux, the moisture vapor transmission properties of the polymer used to form the top layer, and the need for the synthetic skin to be "drapable," i.e. to conform to the contour of the wound bed. In the case of Silastic Medical Grade silicone, a 5-mil thick silicone film layered onto a 50-mil thick layer of collagen-mucopolysaccharide composite is a typical multilayer artificial skin having the desired range of moisture flux. A preferred range for the thickness of the silicone layer is 4-mil to 15-mil, which provides moisture permeability in the range of 0.1 to 2 mg/hr/cm^2, sufficient mechanical strength to allow suturing or stapling and is drapable to allow conformation to wound beds.

The multilayer membranes described herein are useful as dressings for the treatment of burns, cuts, lacerations, abrasions and other such conditions which involve injury or destruction of skin by mechanical, thermal, chemical or other external insult by local or systemic disease. Also, the membranes themselves can be used as artificial grafts wherein they temporarily replace functions of normal skin and provide a template for permanent cellular regeneration.

The invention is further and more specifically illustrated by the following examples.

Example 1 - Preparation of the Artificial Skin

Artificial skin was produced according to the procedure described in Yannas *et al.*, U.S. Patent No. 4,947,840. The primary modifications to this procedure, aside from scaling up the size of the batch, are the use of bovine tendon collagen rather than bovine hide collagen, and that

black threads were embedded in the silicone layer for ease of identification.

Example 2 - Efficacy Of Perforated Artificial Skin Versus Unperforated Artificial Skin

Patients Chosen for the Study

Twenty patients were enrolled in the study. All patients were required to complete a minimum of 12 months of study following healing of the epidermal autograft, as well as to have procedurally correct case reports.

Eligibility was determined in part by age and burn size. Patients of any age up to age 70 were eligible for entry into the study according to the following sliding scale:

AGE	BURN SIZE
less than 50 years	Any burn Size
50-59 years	40% or less TBSA
60-69 years	30% or less TBSA
70 years and older	Not Eligible

TBSA is the total body surface area

Males and nonpregnant females were allowed entry into this study. In addition, patients were chosen who had thermal burn injuries which were, as judged by the investigator, deep partial-thickness or full-thickness wounds and amenable to excisional therapy, had been hospitalized within 48 hours of burn injury and in whom excision of eschar started within 7 days of burn injury and completed within 21 days after the injury.

Patients who had significant concomitant disease, electrical or chemical burns, were pregnant, had wounds infected to a clinically significant degree, or had wounds previously treated by excisional therapy were excluded from the study.

Application of the perforated artificial skin was begun within seven days of injury and completed within 21 days of injury. Patients entered this clinical trial with full-thickness or deep partial-thickness injuries requiring excision. Table 1 describes the percentage of total body surface area (TBSA) and depth (partial-thickness, full-thickness and total) of the wound for all patients.

Table 1.
Burn Severity By Depth And By Surface Area
All Study Patients
N = 20

% of Total Body Surface Area	Partial-Thickness n(%) †	Full-Thickness n (%) †	Total Area Burned n (%) †
0 - 10	8 (40)	4 (20)	0 (0)
11 - 20	3 (15)	4 (20)	0 (0)
21 - 30	4 (20)	1 (5)	1 (5)
31 - 40	1 (5)	3 (15)	4 (20)
41 - 50	2 (10)	3 (15)	7 (35)
51 - 60	1 (5)	2 (10)	1 (5)
61 - 70	1 (5)	1 (5)	2 (20)
71 - 80	0 (0)	2 (10)	2 (20)
81 - 90	0 (0)	0 (0)	3 (15)
91 - 100	0 (0)	0 (0)	0 (0)
Mean ± SD	19.1 ± 19.9	35.4 ± 22.4	53.6 ± 19.4
Median	15.5	32.7	46.0
Range	0 - 66	0 - 78.0	30 - 90

SD = Standard Deviation
† = % TBSA data by row do not sum to 100%, reflecting the distribution of TBSA injury across all patients

Procedure for Applying the Artificial Skin

The artificial skin of Example 1 was thoroughly rinsed in sterile normal saline prior to application. The rinsing

procedure was to soak one of the artificial skin devices in sterile, pyrogen free, normal saline solution for 10 minutes, changing the solution twice.

For some wound sites the artificial skin was cut with a Collins ampligraft with a 2:1 meshing ratio, similar to Figure 2, to give a slitted artificial skin capable of being expanded to cover twice its normal area. In this study, however, the artificial skin was not expanded.

The wound was then excised to the level of viable tissue. The excisional techniques used for the artificial skin sites were fascial, sequential, or tangential. It is critical to the successful take of the artificial skin that excision be complete and that no eschar remain.

Complete hemostasis was also achieved before application of the artificial skin by fine needle point cauterization and application of topical epinephrine at concentration of 1:10,000. The presence of hematoma will cause loss of the artificial skin take in the affected area. Broad area cauterization that could decrease wound bed viability was avoided.

The artificial skin was accurately shaped to fit the excised wound margins to minimize scarring at these margins; it was not allowed to overlap onto nonexcised areas or onto other sheets of the artificial skin. The artificial skin was cut with sterile scissors by placing the sheet of artificial skin over the open area and cutting it exactly to the edge of the wound.

The artificial skin was applied to the wound so that the collagen template layer was in direct contact with the excised wound. The silicone layer (identified by the black threads) was placed *out* (away from the wound bed). The material readily adhered and conformed to the wound surface. Any air bubbles were carefully removed by moving them to the edge of the sheet. The artificial skin sheets were secured by staples or sutures placed in an interrupted

fashion (with fine synthetic monofilament suture, or 4/0 or 5/0 chromic, using a fine atraumatic needle) under slight tension. Care was taken not to spread or expand the membrane and to achieve a primary closure between the artificial skin and adjacent unburned skin or between sheets of the artificial skin. Each strip of artificial skin was sutured or stapled in place independently.

The area was covered with an inner dressing consisting of a single layer of wide mesh gauze, secured by staples or sutures to the normal tissue at the edges of the grafted area. This layer was then wrapped with an outer dressing consisting of two or three layers of 4 inch (10.2 cm) wide rolled gauze.

Postoperative Care

The postoperative care followed a similar protocol to that used following treatment with full sheet or meshed autograft. Dressings were inspected daily for evidence of infection. The patient was also monitored for evidence of sepsis.

The outer dressing were changed every 4 to 5 days. The inner dressing was not disturbed unless there were problems requiring intervention. The attachment of the silicone layer was also examined. Fluid accumulation was treated by excising the silicone layer over the affected area.

The silicone layer was removed after the collagen layer had been replaced by neodermis, usually 14-21 days after grafting using forceps. After removal of the silicone layer, a thin layer of meshed epidermal autograft (0.002 to 0.005 inches) was applied to the artificial skin neodermis by conventional techniques.

Statistical Methodology

Descriptive statistics are given for all entry, treatment, and outcome characteristics. Frequencies and confidence intervals were used to summarize the infection and culture results. Percentages were used to summarize physician assessments at each follow-up visit.

Confidence intervals for dichotomous data were computed using the binomial distribution. The method of Bickel and Doksum, *Mathematical Statistics - Basic Ideas and Selected Topic*, Holden-Day, San Francisco, 180-2 (1977), was used for the calculations. Confidence intervals for continuous data were computed for both the mean and the median. Confidence intervals for the median were based on the Sign Test (Hollander and Wolfe, *Nonparametric Statistical Methods*, Wiley, New York, 48-9 (1976)).

Because of the skewed nature of the artificial skin take and epidermal-autograft take variables, nonparametric statistics were used to analyze effect on take in all comparisons. The Kruskal-Wallis test SAS/STAT User's Guide, SAS Institute, Inc., Cary, North Carolina 27513, was used to test for differences in both the artificial skin take and epidermal-autograft take among anatomic locations. The Kruskal-Wallis test was also used to test for significance of day of excision on the artificial skin take. The maximum-likelihood, chi-square test was used to test for differences in poor take of the artificial skin ($\leq$10%) among anatomic locations.

Results

Table 2 gives the distribution of the artificial skin take over the 56 wound sites for which take was fully evaluated. Overall the take of the artificial skin to freshly excised burn injuries was greater than 80% in 41 of the 56 (73.2%) wound sites. Twenty-nine (52%) of the wound sites had 100% artificial skin take. Conversely, 8 of the

56 (14%) wound sites had 10% or less take. Five wound sites (9%) had 0% take. For all sites in total the mean take was 80.6% and the median take was 100%.

When comparing the perforated artificial skin to the sheet artificial skin, perforated artificial skin had greater than 80% take in 28 of the 34 (82%) wound sites. Sites receiving sheet artificial skin had greater than 80% take in only 13 of the 22 (59%) wound sites studied. Twenty sites (59%) treated with perforated artificial skin had 100% take, whereas nine sites (41%) that received full sheet artificial skin had 100% take. Both the full sheet and perforated artificial skin had 0% take in 9% of the sites evaluated. The mean take for perforated and full sheet were 85% and 74%, respectively. The medians were 100% for perforated and 95% for full sheet artificial skin.

Table 2.
Artificial Skin Take
N = 20

Take	Meshed Frequency	%	Sheet Frequency	(%)	Total Frequency %	
0-11	4	(12)	4	(18)	8	(14)
11-20	0	(0)	1	(5)	1	(2)
21-30	0	(0)	0	(0)	0	(0)
31-40	0	(0)	0	(0)	0	(0)
41-50	1	(3)	0	(0)	1	(2)
51-60	0	(0)	0	(0)	0	(0)
61-70	1	(3)	0	(0)	1	(2)
71-80	0	(0)	4	(18)	4	(7)
81-90	2	(6)	0	(0)	2	(4)
91-100	26	(76)	13	(59)	39	(70)
TOTAL	34	(100)	22	(100)	56† (100)	
Mean ± SD	85.0 ± 32.2		73.8 ± 38.7		80.6 ± 35.0	
Median	100		95		100	
Range	0-100		0-100		0-100	

† Patient 34-8 (three wound sites) died before take was assessed.
SD = Standard Deviation

Sixteen of 56 (29%) were reported as having fluid accumulation under the artificial skin. Ten of 22 sheet sites (46%) had fluid accumulation reported, but only 6 of 34 meshed sites (18%) had fluid accumulation.

Table 3 indicates the assessment of cultures taken on the day of epidermal autografting and the assessment of clinical infection based on those culture results for the 36 sites with culture data on that day. For the pooled perforated and sheet artificial skin data, positive cultures were found in 30 wound sites (83%). Two (6%) were reported as clinically significant. Fifteen (75%) of the perforated artificial skin sites had positive cultures immediately postexcision, none of the perforated artificial skin sites were reported to be clinically significant. Fifteen (94%) of the sheet artificial sites had positive cultures immediately postexcision, two sites (12%) were reported to be clinically significant. The perforated artificial skin appears to have a lower percentage of positive cultures than the sheet artificial skin. Also, none of the perforated artificial skin were reported to have significant clinical infections, whereas two sheet artificial sites were reported to have clinical infections.

Table 3

Cultured Results on the Day of Epidermal Autograft
All Study Patients
N = 20

	Meshed N = 22 sites		Sheet N = 16 sites		Total N = 38 sites		
Source	Freq	(%)	Freq	(%)	Freq	(%)	95% Cl
Positive Culture	15	(75)	15	(94)	30	(83)	(72, 95)
Clinically Significant	0	(0)	2	(12)	2	(6)	(0, 14)

Cl = Confidence Interval
References: Supporting Data for the Meshed vs. Sheet INTEGRA Study, Data Listings 4A, page 06-0213 and 7B, page 06-0281

Wounds considered to be suspiciously infected by visual observation were also cultured. Table 4 shows the results of cultures obtained after the day of excision and before the day of the epidermal autograft. If there were repeated observations on a single day, only the last determination was used in this analysis. There were 98 additional cultures obtained after the day of excision and before the day of epidermal autograft used in the analysis. Of those, 88 (90%) were positive cultures and 33 (34%) were considered clinically significant (Table 4). The meshed artificial skin appeared to have a lower percentage of sites with positive cultures (85% vs. 94% for sheet sites) as well as a lower percentage of sites with clinically significant culture results (14% vs. 55%).

Table 4

Cultured Results After Excision and
Before Epidermal Autograft
All Study Patients
N = 20

	Meshed N = 51 sites		Sheet N = 47 sites		Total N = 98 sites		
Source	Freq	(%)	Freq	(%)	Freq	(%)	95% Cl
Positive Culture	44	(86)	44	(94)	88	(90)	(84, 96)
Clinically Significant	7	(14)	26	(55)	33	(34)	(25, 43)

Cl = Confidence Interval

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described specifically herein. Such equivalents are intended to be encompassed in the scope of the following claims.

CLAIMS

1. A method of preventing or reducing infection at a wound site (e.g., a burn wound) which is undergoing repair with a synthetic skin graft (e.g., a porous biodegradable polymeric multilayer membrane) comprising providing a moisture control layer disposed on the skin graft which is perforated such that when the bottom layer of the skin graft is applied to a wound, the moisture control layer is permeable to fluid in the presence of hydrostatic pressure from exudate in the wound and substantially impermeable to fluid and water vapor in the wound in the absence of hydrostatic pressure from exudate in the wound.

2. A method of covering a burn or wound site on a human or animal, comprising applying to a burn or wound a multilayer membrane (e.g., two layers) comprising a porous biodegradable polymeric membrane layer having disposed thereon a moisture control layer that is perforated such that when the bottom layer of the multilayer membrane is applied to a wound, the membrane is permeable to fluid in the presence of hydrostatic pressure from exudate in the wound and substantially impermeable to fluid and water vapor in the wound in the absence of hydrostatic pressure from exudate in the wound.

3. A multilayer membrane, comprising a porous biodegradable polymeric membrane layer having disposed thereon a moisture control layer that is perforated such that when the bottom layer of the multilayer membrane (e.g., two layers) is applied to a wound, the membrane is permeable to fluid in the presence of hydrostatic pressure from exudate in the wound and substantially

substantially impermeable to fluid and water vapor in the wound in the absence of hydrostatic pressure from exudate in the wound.

4. Synthetic skin, comprising a porous biodegradable polymeric membrane layer having disposed thereon a moisture control layer that is perforated such that when the bottom layer of the synthetic skin is applied to a wound, the synthetic skin is permeable to fluid in the presence of hydrostatic pressure from exudate in the wound and substantially impermeable to fluid and water vapor in the wound in the absence of hydrostatic pressure from exudate in the wound.

5. The multilayer membrane, synthetic skin or method according to any one of Claims 1 to 4 wherein the polymeric layer has a pore size from between about 9 μm and about 630 μm (e.g., between about 20 μm and about 200 μm) and a pore volume fraction of greater than about 80%.

6. The multilayer membrane, synthetic skin or method according to any one of Claims 1 to 5 wherein the moisture control layer is perforated so that moisture loss from a wound to which the bottom layer of the multilayer membrane has been applied is maintained below about 2.2 mg/cm^2/hour.

7. The multilayer membrane, synthetic skin or method according to any one of Claims 1 to 6 wherein the polymeric layer comprises crosslinked collagen, a crosslinked collagen-glycosaminoglycan composite, or unbanded collagen-glycosaminoglycan.

8. The multilayer membrane, synthetic skin or method according to any one of Claims 1 to 7 wherein the moisture control layer is formed from a synthetic polymer selected from the group consisting of silicone resins, polyurethane, polyacrylate esters, polymethacrylate esters and polyurethanes.

9. The multilayer membrane, synthetic skin or method according to any one of Claims 1 to 8 wherein the perforations additionally penetrate the polymeric membrane layer or the skin graft.

10. The multilayer membrane, synthetic skin or method according to any one of Claims 1 to 9 wherein (i) the perforations comprise a plurality of slits (e.g., the slits are parallel and arranged in aligned or staggered rows), (ii) the perforations comprise a plurality of cross slits arranged in parallel rows, or (iii) the perforations comprise a plurality of holes arranged in a trigonal pattern.

11. The multilayer membrane, synthetic skin or method of Claim 10 wherein the moisture control layer has (i) at least two perpendicular continuous bands of membrane which the slits do not intersect, thereby preventing expansion of the membrane upon application of a force along the continuous bands of membrane, (ii) parallel, lateral axis such that at least one slit intersects each parallel, lateral axis, thereby allowing expansion of the membrane upon application of a force along the lateral axis, or (iii) a sufficient number of slits intersects each parallel lateral axis such that the membrane can expand to about twice its original area upon application of force along the lateral axis.

12. A multilayer membrane, synthetic skin or method according to any one of Claims 1 to 12 comprising a polymeric membrane layer which: (1) has controllable degradability in the presence of body enzymes; (2) has controllable solubility in the presence of body fluids; (3) is substantially nonimmunogenic upon grafting or implantation; (4) provokes no substantial foreign body response upon grafting or implantation; (5) and, promotes the adherence of cells, such as fibroblasts and endothelial cells.

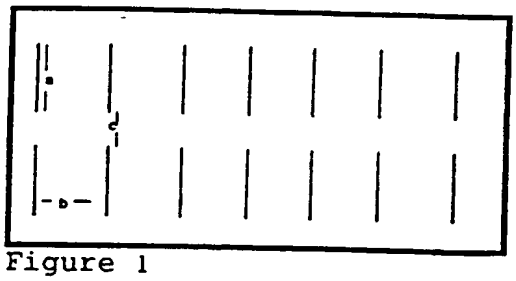

Figure 1

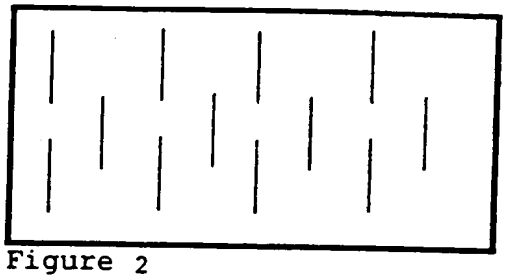

Figure 2

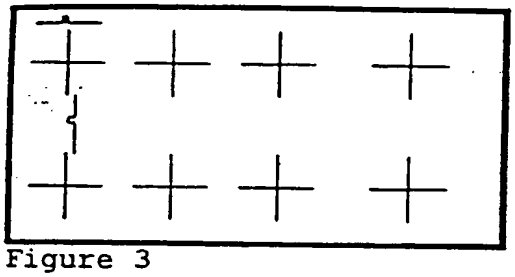

Figure 3

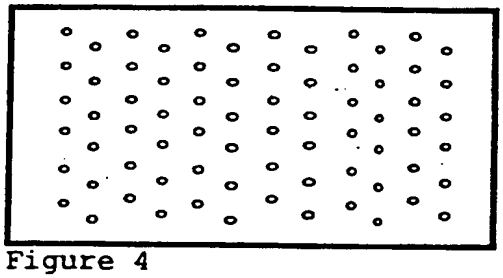

Figure 4

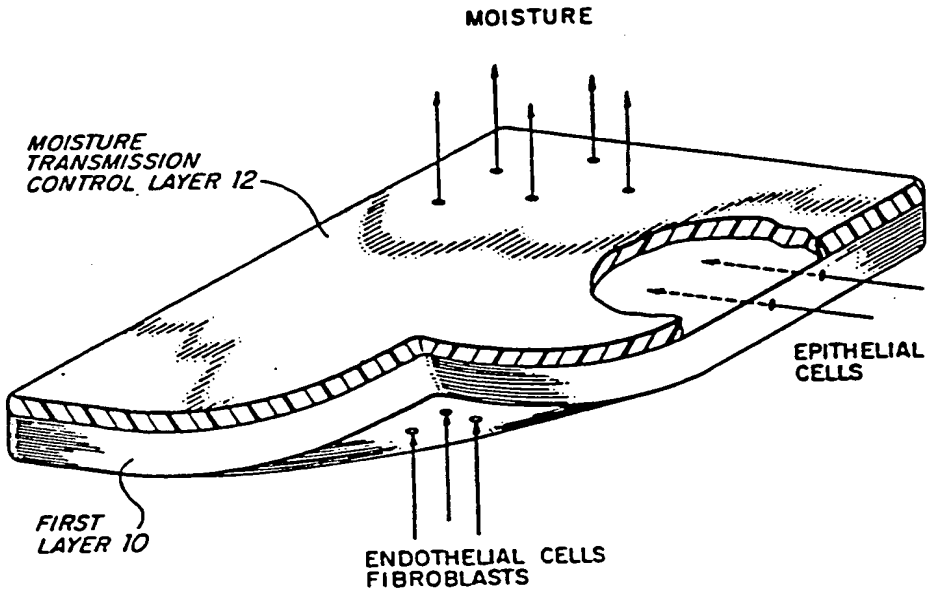

Figure 5

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 96/13244

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61L27/00 A61F2/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61L A61F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP,A,0 462 426 (FIDIA SPA) 27 December 1991 see claims; examples 1-3	1-12
Y	US,A,4 060 081 (YANNAS IOANNIS V ET AL) 29 November 1977 cited in the application see claims; figure	1-12
P,Y	US,A,5 489 304 (ORGILL DENNIS P ET AL) 6 February 1996 see column 2, line 15 - line 37; claims	1-12
A	EP,A,0 399 782 (MINNESOTA MINING & MFG) 28 November 1990 see claims	1-12

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
20 January 1997	04.02.97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	ESPINOSA, M

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SURGERY, vol. 103, no. 4, April 1988, pages 421-431, XP002023073 S.T. BOYCE ET AL.: "BIOLOGIC ATTACHMENT, GROWTH, AND DIFFERENTIATION OF CULTURED HUMAN EPIDERMAL KERATINOCYTES ON A GRAFTABLE COLLAGEN AND CHONDROITIN-6-SULFATE SUBSTRATE" see abstract -----	1-12

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 96/13244

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 1-2
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 1 and 2 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compounds.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 96/13244

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0462426	27-12-91	IT-B-	1248934	11-02-95
		AU-B-	637235	20-05-93
		AU-A-	7806691	05-12-91
		CA-A-	2043527	02-12-91
		JP-A-	4231061	19-08-92
		US-A-	5326356	05-07-94
US-A-4060081	29-11-77	CA-A-	1071814	19-02-80
		DE-A-	2631909	10-02-77
		FR-A-	2332863	24-06-77
		GB-A-	1518748	26-07-78
		JP-C-	1136044	28-02-83
		JP-A-	52038796	25-03-77
		JP-B-	57027834	12-06-82
US-A-5489304	06-02-96	NONE		
EP-A-0399782	28-11-90	CA-A-	2015495	23-11-90
		JP-A-	3004848	10-01-91

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : **A61L 15/40, 15/22, 27/00, C12N 5/00**

A3

(11) International Publication Number: **WO 99/00151**

(43) International Publication Date: 7 January 1999 (07.01.99)

(21) International Application Number: PCT/GB98/01882

(22) International Filing Date: 26 June 1998 (26.06.98)

(30) Priority Data:

9713406.8	26 June 1997 (26.06.97)	GB
9725209.2	28 November 1997 (28.11.97)	GB

(71) Applicant *(for all designated States except US)*: SMITH & NEPHEW PLC [GB/GB]; 2 Temple Place, Victoria Embakment, London WC2R 3BP (GB).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: THOMSON, Brian, Mark [GB/GB]; 33 Burnby Lane, Pocklington, York YO42 2QE (GB). ALI, Saad, Abdul, Majeed [GB/GB]; 71 Yarburgh Way, York YO10 5HQ (GB). MEDCALF, Nicholas [GB/GB]; 12 Clayfield Close, Pocklington, York YO42 2PU (GB). MALTMAN, John [GB/GB]; 12 Lundy Close, Waterside Park, Clifton, York YO30 5GQ (GB). WINTER, Sharon, Dawn [GB/GB]; 12 Lundy Close, Waterside Park, Clifton, York YO30 5GQ (GB).

(74) Agent: SMITH & NEPHEW GROUP RESEARCH CENTRE; Group Patents & Trade Marks Dept., York Science Park, Heslington, York YO10 5DF (GB).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(88) Date of publication of the international search report:
25 March 1999 (25.03.99)

(54) Title: CELL CULTURE PRODUCTS

(57) Abstract

A wound dressing which comprises a carrier layer having a non–adherent to cell layer on a wound facing surface thereof. The non–adherent layer has bonded thereto a biodegradable cell anchoring layer which anchors mammalian cells. In use, the degradable layer breaks down releasing the cells into the wound site which are discouraged from reattaching to the dressing by the non–adherent layer. Thus the dressing can switch from a cell binding state to a state in which the binding of cells is discouraged. Systems, methods of treatment and methods of manufacturing the dressing are also disclosed.

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 98/01882

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61L15/40 A61L15/22 A61L27/00 C12N5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 06837 A (INTEGRA LIFESCIENCES CORP) 27 February 1997	1,2
Y	see page 1, line 12 - line 24	3,17-19
A	see page 2, line 9 - line 20	4-9, 12-14
	see page 8, line 7 - line 17	
	see page 12, line 29 - page 13, line 17	

	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 January 1999	19/01/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Gundlach, B

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 97 06835 A (SMITH & NEPHEW ;RICHARDSON MARK CHRISTOPHER (GB); BLOTT PATRICK LE) 27 February 1997 cited in the application	3,17-19
A	see page 3, paragraph 2 see page 5, line 3 - line 9 see page 7, paragraph 3 see page 11, paragraph 1 see page 12, paragraph 1 see page 17; claims 1,5,9,16-19,22	1,2,5, 12-15
X	US 4 060 081 A (YANNAS IOANNIS V ET AL) 29 November 1977	1,2
A	see column 6, line 10 - column 7, line 44 see column 13, line 47 - column 14, line 4	3-7, 12-16
A	US 5 410 016 A (HUBBELL JEFFREY A ET AL) 25 April 1995 see column 4, line 29 - line 39 see abstract; claims 1,19	1-8,10, 11

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 98/01882

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9706837	A	27-02-1997	AU	6775596 A	12-03-1997
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			CA	2226747 A	27-02-1997
US 4060081	A	29-11-1977	CA	1071814 A	19-02-1980
			DE	2631909 A	10-02-1977
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			JP	1136044 C	28-02-1983
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US 5410016	A	25-04-1995	US	5380536 A	10-01-1995
			US	5468505 A	21-11-1995
			US	5626863 A	06-05-1997
			US	5567435 A	22-10-1996
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			DE	69126535 D	17-07-1997
			DE	69126535 T	25-09-1997
			EP	0553195 A	04-08-1993
			ES	2104727 T	16-10-1997
			WO	9206678 A	30-04-1992
			US	5462990 A	31-10-1995
			US	5820882 A	13-10-1998
			US	5627233 A	06-05-1997
			US	5567440 A	22-10-1996
			US	5232984 A	03-08-1995
			US	5849839 A	15-12-1998

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5WO
09/446379

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
MLC/CLC/2139PC		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB98/01882	26/06/1998	26/06/1997
International Patent Classification (IPC) or national classification and IPC A61L15/00		
Applicant SMITH & NEPHEW PLC et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
21/12/1998	11.10.99
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Gore, V Telephone No. +49 89 2399 8590

Form PCT/IPEA/409 (cover sheet) (January 1994)

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB98/01882

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-16 as originally filed

Claims, No.:

1-19 as originally filed

Drawings, sheets:

1/11-11/11 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 18.

because:

☒ the said international application, or the said claims Nos. 18 relate to the following subject matter which does not require an international preliminary examination (*specify*):

see separate sheet

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-19
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-19
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-17,19 (YES), 18 see separate sheet
	No:	Claims	

2. Citations and explanations

see separate sheet

1. Reference is made to the following document :

   D1 : WO-A-9706835

Regarding point III

2. Claim 18 relates to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

Regarding point V

3. For the assessment of the present claim 18 on the question whether it is industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

4. D1 discloses a wound dressing comprising a conformable carrier having a wound-facing surface to which a layer of cultured mammalian cells is anchored, the carrier comprising a synthetic polymer layer which has a water uptake of at least 16 % w/w and is non-inhibitory to cell growth (cl.1). In an embodiment, the carrier comprises two synthetic polymer layers, at least one of which has the technical features described in cl.1 (cl.5). Optionally, the wound-facing surface of the carrier is treated so as to reduce the contact angle of said surface with the skin/wound (cl.9-10). A suitable surface treatment is corona discharge treatment (see page 5 §2). Suitable synthetic polymers for the carrier are polyurethanes, polyetherpolyesters,

polyacrylamides and polyethylene oxides. A film made of one of these polymers may additionally be coated with a material such as ethylene vinyl acetate which allows attachment of anchorage-dependent mammalian cells (see page 7 §3). The mammalian cells are preferably keratinocytes (see page 11 §1).
Neither D1 nor any other available prior art discloses a wound dressing comprising a carrier layer having a wound-facing surface that is non-adherent to anchorage-dependent cells and having a biodegradable cell-anchoring layer disposed thereon.
Claims 1-16 seem to be new.

4.1 Since the wound dressing of claims 1-16 seems to be new, a process for manufacturing said wound dressing (cl.19) and its use in a cell culture system (cl.17) or in a method for treating a skin trauma (cl.18) would also be new.

5. D1, considered as the closest prior art, solves the problem of providing a conformable carrier for growing a cell layer that can be readily transferred to a wound surface. D1 focuses on finding a material suitable for anchoring the cells (see page 5). The present application, on the other hand, seeks to provide a wound dressing that becomes non-adherent to cells following the application to a wound, so that the transfer of the cells (that may be anchored to said dressing) to the wound is facilitated. The wound dressing of D1 does not exhibit such properties because the cells are strongly anchored to the cell-adherent polymer of the wound dressing.
The technical problem solved by the present application is not mentioned in the prior art. The prior art does not describe either a wound dressing that is likely to have the same properties as the wound dressing defined in D1 of the application. Consequently, claims 1-19 appear to involve an inventive step.